CLAIMS

What is claimed is:

1. A method of identifying ligands that are internalized into a cell, said method comprising:

i) contacting said cell with a reporter non-covalently coupled to a ligand;

ii) dissociating the reporter from the ligand and removing dissociated reporter from the surface of said cell; and

iii) detecting the presence of the reporter within said cell, whereby the presence of the reporter within said cell indicates that said ligand is internalized into said cell.

2. The method of claim 1, wherein said contacting comprises contacting said cell with a ligand comprising an epitope tag and contacting said cell with a reporter comprising a moiety that binds said epitope tag.

3. The method of claim 1, wherein said ligand is a ligand that binds to a cell surface receptor.

4. The method of claim 1, wherein said ligand is a peptide.

5. The method of claim 1, wherein said ligand is selected from the group consisting of an scFv, an Fv, an Fab, monoclonal antibody, a cytokine, and a growth factor.

6. The method of claim 1, wherein said ligand is a ligand produced in a phage display library.

7. The method of claim 6, wherein said phage display library uses a filamentous phage.

8. The method of claim 1, wherein said reporter is non-covalently coupled to a ligand by an epitope tag.

9. The method of claim 1, wherein said reporter is non-covalently coupled to a ligand by an epitope tag selected from the group consisting of a His-tag, a Flag-tag, an HA-tag, a myc-tag, and a DYKDDDDK epitope.

10. The method of claim 1, wherein said reporter is a reporter selected from the group consisting of an enzyme, a colorimetric label, a fluorescent label, a luminescent label, a radioactive label, a nanoparticle, and a liposome.

11. The method of claim 1, wherein said epitope tag is a hexahistidine (His_6) tag and said reporter is a liposome comprising a nitrilotriacetic acid (NTA) lipid.

12. The method of claim 1, wherein said ligand is an antibody and said epitope tag is attached to said antibody through a covalent linkage to protein A.

13. The method of claim 1, wherein said cell is a cancer cell.

14. The method of claim 1, further comprising isolating the ligand that is internalized into said cell.

15. The method of claim 14, wherein said isolating comprises determining the amino acid sequence of a ligand that is internalized by said cell or determining the sequence of a nucleic acid encoding said ligand.

16. A method of screening a cell for a receptor that internalizes a ligand, said method comprising:

i) contacting said cell with an effector non-covalently coupled to a ligand;

ii) dissociating the effector from the ligand and removing dissociated effector from the surface of said cell; and

iii) detecting the presence of the effector within said cell, whereby the presence of the effector within said cell indicates that said cell internalizes said ligand.

17. The method of claim 16, wherein said ligand is a ligand known to be internalized by a cell.

18. The method of claim 16, wherein said ligand is a member of a library of ligands.

19. The method of claim 18, wherein said library of ligands comprises at least 1000 different members.

20. The method of claim 16, wherein said ligand is a peptide.

21. The method of claim 16 wherein said ligand is selected from the group consisting of an scFv, an Fv, an Fab, monoclonal antibody, a cytokine, and a growth factor.

22. The method of claim 16, wherein said ligand is a ligand produced in a phage display library.

23. The method of claim 22, wherein said phage display library uses a a filamentous phage.

24. The method of claim 16, wherein said effector is non-covalently coupled to a ligand by an epitope tag.

25. The method of claim 16, wherein said effector is non-covalently coupled to a ligand by an epitope tag selected from the group consisting of a His-tag, a Flag-tag, an HA-tag, a myc-tag, and a DYKDDDDK (SEQ ID NO:1) epitope.

26. The method of claim 16, wherein said effector is a reporter selected from the group consisting of an enzyme, a colorimetric label, a fluorescent label, a luminescent label, a radioactive label, a nanoparticle, and a liposome.

27. The method of claim 16, wherein said epitope tag is a hexahistidine (His_6) tag and said effector is a liposome comprising a nitrilotriacetic acid (NTA) lipid or an iminodiacetic acid (IDA) lipid.

28. The method of claim 16, wherein said ligand is an antibody and said epitope tag is attached to said antibody through a covalent linkage to protein A or protein G.

29. The method of claim 16, wherein said cell is a cancer cell.

30. The method of claim 29, wherein said cell is a cell known to overexpress a receptor.

31. The method of claim 16, further comprising isolating the cell that internalizes said ligand..

32. A ligand library comprising a plurality of members said members comprising ligands and epitope tags where the ligands vary between members of the library and the epitope tags are constant.

33. The ligand library of claim 32, wherein said ligands are non-covalently coupled to reporters through the epitope tags.

34. The library of claim 33, comprising at least 10^5 different ligands.

35. The library of claim 33, wherein said ligands are peptides.

36. The method of claim 33, wherein said ligands are selected from the group consisting of an scFv, an Fv, an Fab, monoclonal antibody, a cytokine, an enzyme, a hormone, and a growth factor.

37. The method of claim 33, wherein said ligands are ligands produced in a phage display library.

38. The method of claim 37, wherein said phage display library uses a filamentous phage.

39. The library of claim 33, wherein said reporters are non-covalently coupled to said ligands by an epitope tag selected from the group consisting of a His-tag, a Flag-tag, an HA-tag, a myc-tag, and a DYKDDDDK (SEQ ID NO:1) epitope.

40. The library of claim 33, wherein said reporters are reporters selected from the group consisting of an enzyme, a colorimetric label, a fluorescent label, a luminescent label, a radioactive label, a nanoparticle, and a liposome.

41. The library of claim 33, wherein said epitope tag is a hexahistidine (His_6) tag and said reporters are liposomes comprising a nitrilotriacetic acid (NTA) lipid or an iminodiacetic acid (IDA) lipid.

42. The library of claim 33, wherein said ligands are antibodies and said epitope tags are attached to said antibodies through a covalent linkage to protein A or protein G.

43. The library of claim 33, wherein members of said library are polyvalent for said ligands.

44. A construct for screening a cell for an internalizing receptor, said construct comprising a ligand non-covalently coupled to an effector through an epitope tag.

45. The construct of claim 44, wherein said ligand is a peptide.

46. The construct of claim 44, wherein said ligand is selected from the group consisting of an scFv, an Fv, an Fab, monoclonal antibody, a cytokine, an enzyme, a hormone, and a growth factor.

47. The construct of claim 44, wherein said ligand is a ligand produced in a phage display library.

48. The method of claim 47, wherein said phage display library uses a filamentous phage.

49. The construct of claim 44, wherein said reporter is non-covalently coupled to said ligands by an epitope tag selected from the group consisting of a His-tag, a Flag-tag, an HA-tag, a myc-tag, and a DYKDDDDK (SEQ ID NO:1) epitope.

50. The construct of claim 44, wherein said reporter a reporter selected from the group consisting of an enzyme, a colorimetric label, a fluorescent label, a luminescent label, a radioactive label, a nanoparticle, and a liposome.

51. The construct of claim 44, wherein said epitope tag is a hexahistidine (His_6) tag and said reporter is a liposome comprising a nitrilotriacetic acid (NTA) lipid lipid or an iminodioacetic acid (IDA) lipid.

52. The construct of claim 44, wherein said ligand is an antibodies and said epitope tag is attached to said antibody through a covalent linkage to protein A or protein G.

53. The construct of claim 44, wherein said construct is polyvalent for said ligand.

54. A kit for identifying an internalizing cell or for screening a ligand that is internalized by a cell, said kit comprising a container containing a ligand library of any one of claims 32 through 43.

55. The kit of claim 54, further comprising instructional materials teaching the use of said library to identify a cell that internalizes a ligand or to identify a ligand that is internalized by a cell.

56. A method of identifying internalizing receptors, said method comprising:

i) contacting a cell with a reporter non-covalently coupled to a ligand;

ii) dissociating the reporter from the ligand and removing dissociated reporter from the surface of said cell;

iii) detecting the reporter within said cell, if said reporter is present within said cell, whereby the presence of the reporter within said cell indicates that said ligand binds to an internalizing receptor and is internalized;

iv) identifying or recovering the ligand bound to the reporter within said cell; and

v) identifying a receptor that binds to said ligand.

57. The method of claim 56, wherein said identifying a receptor is by affinity chromatography or immunohistochemistry.

58. The method of claim 56, further comprising entering the identity of said receptor into a database of internalizing receptors.

59. A method of screening an agent for the ability to modulate internalization of a ligand into a cell, said method comprising:

i) contacting said cell with a reporter non-covalently coupled to a ligand known to be internalized by said cell;

ii) contacting said cell with a test agent;

iii) dissociating the reporter from the ligand and removing dissociated reporter from the surface of said cell; and

iv) detecting the reporter within said cell, if said reporter is present within said cell, whereby a difference in the amount of reporter internalized by the cell contacted with said test agent as compared to the amount of reporter internalized by said cell when contacted with a lower concentration of said test agent indicates that said test agent modulates the internalization of said ligand by said cell.

60. The method of claim 59, wherein said lower concentration is the absence of said test agent.

61. A method of screening an agent for the ability to modulate internalization of a ligand into a cell, said method comprising:

i) contacting said cell with a first concentration of said agent

ii) contacting said cell with a reporter non-covalently coupled to a ligand known to internalize into said cell;

iii) dissociating the reporter from the ligand and removing dissociated reporter from the surface of said cell; and

iv) detecting the reporter within said cell to obtain a first measurement;

v) contacting said cell with a second concentration of said agent wherein said second concentration is higher that said first concentration;

vi) repeating the steps ii) through iv) to obtain a second measurement; and

vii) comparing the first and the second measurements wherein when the first and the second measurements are different, the agent modulates internalization of said ligand in said cell.

62. A metal chelating lipid comprising a lipid, a hydrophilic polymer, and a metal chelation group attached to said hydrophilic polymer.

63. The metal chelating lipid of claim 62, wherein said chelation group is NTA.

64. The metal chelating lipid of claim 62, wherein said hydrophilic polymer comprises polynucleotide(ethylene glycol).

65. The metal chelating lipid of claim 62, wherein said lipid comprises DSPE.

66. A method of delivering an effector to a cell, said method comprising contacting said cell with:

a metal chelating lipid comprising a lipid, a hydrophilic polymer, and a chelation group attached to said hydrophilic polymer and capable of forming a chelation bond with an epitope tag, and an effector associated with said metal chelating lipid, and

a ligand comprising said epitope tag wherein said cell specifically binds and optionally, internalizes, said ligand.

67. The method of claim 66, wherein said lipid comprises a liposome and said liposome contains or is complexed with said effector.

68. The method of claim 66, wherein said cell is a cancer cell.

69. A composition comprising

a lipid, a hydrophilic polymer, and a chelation group attached to said hydrophilic polymer and capable of forming a chelation bond with an epitope tag;

a ligand comprising said epitope tag where said ligand binds and is optionally internalized by a cell; and

an effector associated with said lipid.

70. The composition of claim 69, wherein said lipid comprises a liposome and said liposome contains or is complexed with said effector.

71. The composition of claim 69, wherein said hydrophilic polymer comprised polynucleotide(ethylene glycol).

72. The composition of claim 69, wherein said lipid comprises DSPE.